THE WALTER AND ELIZA HALL INSTITUTE OF RESEARCH IN PATHOLOGY AND MEDICINE

# THE DIRECTOR'S TWENTY-SEVENTH ANNUAL REPORT 1945-46

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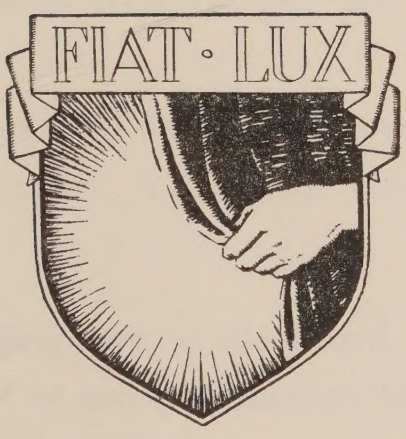


Melbourne:
Spectator Publishing Co., 134a Little Collins St.
1946.

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# History

The Walter and Eliza Hall Institute was founded in 1916 largely on the initiative of the late Sir Harry Allen, then Professor of Pathology in the University of Melbourne. He was associated with members of the medical staff of the Melbourne Hospital in urging the necessity for ensuring that a certain amount of clinical research should be associated with the development of modern diagnostic laboratories in the hospital, then on the Lonsdale Street site. As a result of their representations, the trustees of the late Walter and Eliza Hall agreed to complete the pathological block of the old hospital to provide accommodation for an institute of research in pathology and medicine, and to make annual payments of £2500 towards its upkeep; this was subsequently increased to £3200 annually. It was this contribution of the trustees which allowed the foundation of the Institute, and which has provided the sheet anchor of its support through the years, but ever since its inception the Institute has attracted a widening range of financial support from other official and private sources.

The first director-designate of the Institute was the late Dr. G. C. Mathison, whose death from wounds received on Gallipoli, at the age of 31, ended a career of brilliant promise. The actual work of the Institute was commenced in 1920, under the direction of Dr. S. W. Patterson. He resigned in 1923, and was succeeded by Dr. C. H. Kellaway in August of the same year.

The development of the Institute to its present status will always be linked with Dr. Kellaway's name. For tweny years he was responsible both for the expanding range of scientific work in the laboratories, and for obtaining the necessary financial support for such expansion. During the period of Dr. Kellaway's directorship, and up to the present, the Institute has received substantial help from the University, and from some of the important Victorian charitable trusts, notably the Truby Williams Trust, which has provided £1000 in each of the past two years. From 1934 to 1938 the Rockefeller Foundation gave £1000 per annum to help in the development of the virus department. To continue this work, Mr. E. Alec Cato generously contributed a similar amount for five years, 1939 to 1943.

In addition to this help from private sources an increasing amount of support has been obtained from the Commonwealth Government, at first through the Department of Health, and since 1937 from the National Health and Medical Research Council. This help has been essential to the expansion of the Institute's work.

Other important contributions to the Institute have been the bequests of Mrs. L. E. W. Carty, Mrs. M. M. Mathison, and Mrs. A. M. White, and the donation of £10,000 received from the "Sun News-Pictorial" in 1944.

The culmination of Dr. Kellaway's association with the Institute was the transfer to the present buildings in Parkville. The Committee of Management of The Royal Melbourne Hospital accepted the responsibility for the new building, towards the cost of which generous donations were made by Mr. G. R. Nicholas and the family of the late Mr. A. M. Nicholas, as well as by Mr. Russell Grimwade and others. The occupation of the new Institute was a piecemeal process, carried out during the early years of the war. In 1943 Dr.

Kellaway resigned to take up the post of Scientific Director of the Wellcome Foundation, and Dr. F. M. Burnet was invited to succeed him in March, 1944.

In 1945, the Director became ex-officio Research Professor of Experimental Medicine in the University of Melbourne, and was charged with the development of a University department of epidemiology, funds being made available from the Haley Trust for this purpose.

# The Director's Twenty-Seventh Annual Report

TO THE BOARD

OF THE

## Walter and Eliza Hall Institute of Research in Pathology and Medicine

JULY, 1946.

The most important development of the Institute's work during the year was the initiation of a Clinical Research Unit in The Royal Melbourne Hospital, and the appointment of Dr. Ian J. Wood as Assistant Director in charge of the Unit. This move, which was foreshadowed in the last report, represents our recognition of the responsibility of the Institute to play a full part in the activity of the hospital and teaching school to which it is attached. The proposal was enthusiastically supported by the medical staff of the hospital and by the Medical Superintendent, and has been cordially accepted by the Committee of Management. Within a few weeks of its initiation, the hospital received from each of the Metropolitan Racing Clubs—the Victorian Racing Club, the Victorian Amateur Turf Club, Moonee Valley Racing Club and Williamstown Racing Club—cheques for £5000 for the building and equipping of a new ward in the hospital for the work of the Unit. We are most grateful to the chairmen and committees of these clubs for their magnificent help in this respect.

The staffing of the Clinical Research Unit will be wholly by men and women from the Services. In addition to Dr. Wood, the following appointments have been made—on the clinical side, Dr. W. King as associate physician, and Dr. P. Parsons as senior resident physician; on the laboratory side, Miss M. Freeman as clinical biochemist and Miss L. Limbrick as assistant biochemist. Dr. J. W. Perry will become pathologist to the Unit.

The National Health and Medical Research Council has undertaken the support of the Unit during 1946, supplying £3000 for the purpose. Other assistance has been received from Wyeth

Inc., in the form of a Wyeth Fellowship of £400 p.a., which will be used for the salary of the senior resident physician.

As soon as arrangements could be made, Dr. Wood left to spend four months in America studying clinical research methods. He was most hospitably received at Harvard Medical School and at other centres in U.S. and Canada.

The University Department of Experimental Medicine (Epidemiology) is now functioning actively with the appointment of Dr. F. J. Fenner as Haley Research Fellow.

The development of the Wellcome Library of Epidemiological Research has been delayed owing to building restrictions, but draft plans have been prepared and accepted by the Wellcome Trustees. We hope to go on with the rebuilding necessary in the near future.

Miss J. Scott has been appointed librarian in charge of both the general and the Wellcome libraries.

Collection of Australian epidemiological material is in active progress, and I hope to make arrangements to purchase a considerable number of books while I am in England. The general library has been overhauled, and to some extent reorganised.

It is gratifying to record a continuation of our cordial relationships with American centres of medical research. Joint investigations have been carried out on North Queensland tick typhus with the U.S. Army Medical School, Washington, and on the prevention of measles by gamma globulin with the plasma fractionation unit at Harvard Medical School. We have kept in close touch with the U.S. Army Commission on Influenza, and representative strains of virus from our B and A outbreaks of influenza have been sent to them for comparative study.

An important step in further linking medical research in Australia and America is the award of a National Research Council Fellowship to Dr. B. A. Briody, of Yale Medical School, to be held in this Institute. Dr. Briody hopes to commence work here in July.

During April and May we had as a guest in the Institute, Dr. A. M. Macfarlan, of the staff of the Medical Research Council (Great Britain). He had been investigating an epidemic of poliomyelitis in Singapore, and brought an extensive collection of specimens. It was unfortunate that, owing to the lack of monkeys at the present time, it was impossible to do more than establish two of his strains of virus in animals.

Although he was not a member of the Institute staff, it is fitting that some mention should be made in this report of the sudden and untimely death of Dr. R. J. Wright-Smith, Patho-

logist to the Hospital, on 6th May, 1946. Dr. Wright-Smith was closely associated with the Institute's work for twenty years, and with the development of the Clinical Unit we were looking forward to having the benefit of his experience and his enthusiasm on the pathological side of this work for years to come. The Hospital and the Institute have suffered a heavy loss, and our sincere sympathy goes to his family.

The scientific work of the Institute during the year may be summarised very briefly as follows:

In the Virus Department methods for culturing the virus of mumps in the chick embryo have been developed, and preliminary experiments on human immunization begun. The first recorded Australian epidemic of influenza B (October, November, 1945) was closely studied, and a programme for the study of influenza virus infections in infants initiated. An investigation of certain plasma fractions capable of preventing measles in contacts was carried out on behalf of Dr. Cohn's team at Harvard. Active investigation of the phenomenon of red cell agglutination by viruses has resulted in two findings which may have important theoretical and practical implications, (1) that the haemagglutinin of vaccinia and ectromelia viruses is a complex of virus antigen plus lecithin, and (2) that the action of viruses of the influenza group on human cells may uncover new antigenic groupings on the cell surface.

In the Department of Experimental Medicine a study of the experimental epidemiology of ectromelia of mice has been begun.

The Biochemical Department has been occupied with studies of blood pigment derivatives, of lipids associated with haemagglutination and work on the mechanism of ferment action on sugars.

Apart from a limited amount of work on the serology of oriental schistosomiasis and malaria, the activities of the clinical research section have been only of a preliminary character.

During the year I gave a series of lectures on the background of infectious disease in man for the Melbourne Permanent Post-Graduate Committee, and at the invitation of the New South Wales Post-Graduate Organisation repeated them in Sydney during September. These lectures are being published by the Melbourne Committee.

Other publications by members of the Institute staff during the year, apart from normal scientific papers, were:

"Virus as Organism," by F. M. Burnet. An expansion of the Dunham Lectures given at Harvard in 1944, published by the Harvard University Press.

"Animal Pigments." Annual Review of Biochemistry, 1945, by H. F. Holden.

"The Cultivation of Viruses and Rickettsiae in the Chick Embryo," by W. I. B. Beveridge and F. M. Burnet. Special Report Series, Medical Research Council of Great Britain.

I have accepted an invitation to become a member of the official Commonwealth Delegation to the Empire Science Conferences in London in June and July of this year. This has necessitated the closing of the Annual Report at 30th April, instead of, as usual, 30th June.

I have the honour to record that the Cambridge University has signified that they wish me to accept the Honorary Doctor of Science degree. I shall be able to receive this during my visit to England.

## THE WORK OF THE INSTITUTE

### VIRUS DEPARTMENT

*Mumps.*

No one had succeeded in cultivating the virus which causes mumps until, in 1945, Dr. K. Habel, of the United States National Institute of Health, found a method of growing it in the chick embryo. He inoculated infected human saliva into the parotoid glands of monkeys and subsequently inoculated material from infected glands into the chick embryos. Shortly afterwards, Levens and Enders, of Harvard, published a preliminary report that mumps infected amniotic fluid agglutinated fowl red cells. When these reports came to hand Dr. Beveridge and Miss Lind started a full-time investigation on mumps with Dr. Anderson collecting material from patients. After confirming Habel's work, they showed further that the virus could be isolated in chick embryos directly from human saliva. Three strains were isolated by inoculation into the yolk sac and later, when it was found that the virus could be cultivated more easily by amniotic inoculation, two more were isolated by that method. The saliva was not filtered, but was centrifuged and inoculated along with penicillin and sodium sulphamerazine to suppress bacterial growth.

Investigation of the haemagglutination phenomenon revealed that the virus particles themselves were the active agglutinating agent, as in the case of influenza virus, and that the virus can be eluted subsequently from the red cells. In general, mumps and influenza viruses agglutinate cells from the same species of animal, fowl, human and guinea pig red cells being the most susceptible of those readily available. The mumps virus is weakly

haemolytic for fowl red cells. At low concentrations of the virus, haemagglutination is inhibited by a substance present in allantoic fluid, and in suspensions of yolk sac or chorioallantoic membrane. A suitable technique was developed for titrating antibody in serum by inhibition of haemagglutination, and observations have been made with sera from persons at various intervals after recovery from mumps.

Amniotic and allantoic fluids infected with mumps virus have a slight non-cellular opacity with a bluish opalescence. This appearance is apparently due to the virus particles themselves, as it is removed by procedures which remove the virus, such as high speed centrifugation or absorption with red cells. Allantoic fluids when stored in the refrigerator after inactivation with formalin, usually increase in haemagglutinating titre. This increase, which varies from one batch to another, is commonly twofold in the first two weeks, and a further twofold in the next two weeks. The most probable explanation is that the virus is at first present in aggregates and disaggregation occurs on storage. Various treatments have been tried on fresh virus suspensions, but it has not been found possible to bring about this effect except by storage.

Mumps virus resembles influenza virus in a general way; it is of approximately the same size, its haemagglutinating behaviour is similar, and the manner of its growth in the chick embryo is much the same. However, mumps virus multiplies more slowly than influenza virus, and is more difficult to cultivate in the allantoic cavity. Much time has been spent in investigating the influence of various factors on the amount of virus produced in the allantoic cavity. The optimal conditions for all known factors have been determined in order to devise a method which gives good yields of virus. This point has received great attention because allantoic fluid is the most suitable embryonic component from which to prepare vaccine. It was found impossible to produce a vaccine concentrated by embryonic red cell absorption and elution as with influenza.

Formalin-inactivated virus inoculated subcutaneously in a small group of young adults produced significant amounts of antibody as measured by haemagglutination inhibition. Some showed a considerable antibody response to the first inoculation, and none to the second as is usual with influenza; it is reasonable to assume that these possessed some residual immunity from a past infection either clinical or subclinical. Three others did not respond to the first injection, but did to the second; probably these subjects had no basic immunity, and therefore are representative of the individuals with whom we are mainly concerned in devising an effective immunizing procedure. Further studies on antibody response to vaccines are under way, and limited

amounts have been made available for test in school children against natural infection. The headmasters and medical officers of several boys' boarding schools are co-operating with the Institute in these trials. Two doses of 2 ml. formalin-inactivated allantoic fluid virus are given with an interval of 3-4 weeks between injections. Inoculation of formalinised virus causes an unpleasant sting which is undesirable in children. It has been found that this can be obviated by the addition of 0.25 per cent. phenol to the vaccine. Tests in rabbits showed that the antigenicity of the vaccine was not affected by the phenol.

Small lots of vaccine have been prepared with calcium phosphate as an adjuvant. This substance was found in preliminary experiments by Salk, in U.S.A., to enhance antibody response to influenza vaccine, and it was thought that with mumps it might make possible an effective single dose vaccine. Mumps virus is precipitated with calcuim phosphate, but the preparations so far tried out on members of the laboratory staff have produced rather too severe local reactions for general use.

No convenient *in vivo* test for mumps antibody is available, but neutralisation of the virus can be demonstrated by the inoculation of virus-serum mixtures into the amniotic cavity. With serum from persons recently convalescent from mumps well marked neutralisation occurred. Tests with serum from vaccinated subjects were negative by the usual technique, but a definite effect could be shown if five times the usual amount of fresh, unheated serum were inoculated with about ten minimal infecting doses of virus.

By mixing serum from recently convalescent subjects, or from experimentally immunized rabbits with opalescent high titre fluid, a precipitin reaction could be demonstrated.

*Influenza B Epidemic.*

In October, 1945, an epidemic of influenza B was recognised, first by the appearance of cases in the nursing staff of The Royal Melbourne Hospital, and later in other sections of the community. This was not only the first recorded epidemic due to this virus in Australia, but also appears to have been part of the most extensive world-epidemic of influenza B that has occurred since the influenza viruses were defined. The first reports came from Pacific Island bases during May, 1945. Our October epidemic was followed in November and December by widespread influenza B in United States, and in January and February in England.

Extensive investigations were carried out by Dr. Anderson, Miss Stone and myself on the epidemic as observed in a Melbourne Public School, and, with the collaboration of Dr. Abra-

hams, all cases occurring in the hospital nursing staff were studied.

Features of interest in the epidemic were, first, the relative absence of cases in the Services and in the general adult population, in contrast with its high incidence in schools. Two boarding schools had approximately half their numbers affected over a few weeks. Second, the co-existence of a considerable minority of influenza A cases over the same period. In one school two boys had consecutive attacks of B and A influenza at a 2-3 week interval. This is the first time such an occurrence has been recorded.

The epidemic provided an opportunity to test the effectiveness of the method of isolation described in the last Annual Report. This involves amniotic inoculation of unfiltered throat washings with penicillin and sodium sulphamerazine to prevent or control bacterial infection. The method was highly successful, no difficulty being experienced in isolating A or B virus, provided the washing was obtained within the first 24 hours of fever. The B strains were uniform in character, but differed considerably in antigenic structure from the classical B strain LEE.

*The Serological Response to Influenza B.*

All patients tested showed an antibody rise after infection, but the detailed character of the response varied widely. A large number of tests was made to compare the antibody response to a homologous recently isolated strain and to the standard LEE strain. Two general types of response were noted. Most adults, and some adolescents, gave antibody almost equally active against the epidemic strains and against LEE. A second group, comprising most of the adolescents and a few adults, showed effective antibody against the current strains, but very much less activity against LEE. The results were equally shown with antihaemagglutinin titrations, and with neutralisation tests in the allantoic cavity.

A possible practical implication of these findings is that while most adults may be effectively immunized with any A or B type virus vaccine, success in children, and in a proportion of adults, may demand the use of strictly homologous strains.

*Influenza A Strains from Interepidemic Infections.*

During spring and summer (1944-46) several strains of influenza A were isolated, some during the period of the influenza B epidemic, one in February. These strains have shown distinct differences from those obtained during the 1942 epidemic and in its residuum in 1943. When isolated, all showed well marked O type haemagglutinin reactions, i.e., they failed to agglutinate

fowl cells, but gave relatively high titres with guinea pig, pigeon and human red cells. In 1942 strains there was a rapid change in character on passage to the D-type agglutinating fowl cells to full titre, and only by amniotic passage at limiting dilutions could the O phase be maintained. The recent endemic strains are much less labile. The O form is readily maintained by amniotic passage at moderate dilution, and to obtain a typical D form repeated passage at low dilution is necessary. Of greater interest is the fact that with one strain it has been possible to maintain the O phase by allantoic passage, and to obtain high titre fluids. In this instance, power to grow in the allantoic cavity has developed without the O—D change that was always associated with this development in the A strains previously studied. A further difference now under study is the exceptional ease with which the new A strains are inhibited in antihaemagglutinin titrations by normal human sera.

It remains to be determined whether these strains are characteristic of sporadic influenza A as contrasted with those current in epidemic periods, or whether they are the forerunners of a new type of virus potentially capable of epidemic spread.

*Measles, Varicella, etc.*

A number of unsuccessful attempts were made by Dr. Beveridge and Miss Lind to cultivate the measles virus and demonstrate it in chick tissues by complement fixation.

Dr. Nagler has also been testing out various methods of embryo inoculation in an attempt to cultivate the viruses of herpes zoster and varicella. These methods included the combined use of human tissue cultures with embryo inoculation, but so far no positive results have been obtained.

Dr. Anderson has similarly used a few samples of material from cases of infectious mononucleosis for such attempts, also without success.

*Prevention of Measles with Gamma Globulin.*

At the request of the Harvard team working under Dr. E. J. Cohn on plasma fractionation, Dr. S. G. Anderson, in collaboration with Dr. W. Ket, of the Queen's Memorial Infectious Diseases Hospital, Fairfield, carried out a clinical study of the effectiveness of certain fractions of gamma globulin from pooled human plasma, in preventing measles in children exposed to the disease. The investigation followed standard lines, and the results satisfactorily established the effectiveness of the conventional Fraction II concentrate in preventing or modifying measles. An experimental batch containing globulin derived from "Fraction III" was equally effective.

These results indicate, as might be expected, that measles, as it occurs in this country, responds quite similarly to what has been observed in America.

*The Mechanism of Haemagglutination by Viruses of the Influenza Group.*

Hirst showed, in 1941, that when influenza virus was allowed to react with red cells at 37°C. the initial absorption of the virus and agglutination was followed within an hour or two by the reliberation of the virus, and a more or less complete restabilisation of the cell suspension. Cells so treated were quite insusceptible to agglutination by the virus, and it was assumed that certain cell receptors had been destroyed in the process.

It has previously been shown that the viruses of the influenza group vary in the degree to which they remove these receptors and with the observation that mumps virus is a haemagglutinin active against the same range of cells, further studies along these lines were undertaken. By using appropriate immune sera to stabilise cells from which virus had been almost wholly eluted, it was possible to obtain more satisfactory experimental results and to show a striking regularity in the results. More than a dozen virus strains can be arranged in a linear series such that if a cell suspension after treatment with a virus of the group is agglutinated by virus X, then all the viruses following this one in the series will agglutinate the suspension. Similarly, if the cells are not agglutinated by virus Y then it will also fail to be agglutinated by all viruses which precede Y in the series. In general, if virus Z is used in treating the cells, they become resistant to agglutination by viruses up to and including Z, while they remain sensitive to viruses beyond Z in the series. Sometimes cells may be obtained which are rather more or rather less susceptible than this, but the general linear arrangement of the viruses holds without exception. The series begins with mumps virus and ends with swine influenza virus.

In the course of this work it was observed that after the action of swine virus the cells had become agglutinable by serum from a ferret convalescent from swine influenza. Cells which, as usual, retained a small amount of virus, could only be stabilised by using the smallest effective amount of immune serum; larger amounts of serum removed the virus agglutination, but replaced it by a serum agglutination. Study of this phenomenon showed that both fowl and human cells from which virus had been eluted were agglutinated by a variety of normal sera which were without effect on untreated cells. The effect was a "cold agglutination" and the agent in the serum could be shown to be absorbed in the cold and eluted from washed cells by warming to 37°C.

This phenomenon is at present under study. As far as we are aware it is the first indication reported of cellular change associated with adsorption and elution of influenza viruses. If, as seems likely, the action of the virus is to expose or produce an antigenic grouping not present on the normal cell surface, a number of interesting immunological possibilities arise. The hypothesis on which we are at present working is that the frequent appearance of cold agglutinins in the serum following atypical pneumonia, mumps with orchitis and influenzal pneumonia may be a secondary result of damage to red cells *in vivo* by haemagglutinating viruses.

*The Optimal Method of Titrating Influenza Virus Antibodies.*

Like all workers on the subject, we have found many difficulties in obtaining fully reproducible results in titrations of human or animal immune sera for influenza virus antibodies by haemagglutinating methods. A constant difficulty is the frequent occurrence of inhibition of agglutination by low serum dilutions which by other criteria contain no specific antibody. It has been found that the cells from certain fowls (10-15 per cent. of those tested) are slightly more sensitive than others in direct virus titrations and in serum neutralisation tests give very much lower titres for normal sera, but only slightly reduced titres with immune ones. Control of this variable by using only cells from such selected fowls, and checking with a known negative serum, has made it possible to recognise small amounts of antibody with much more confidence than previously. Apart from the use of these selected fowl cells, the technique now being used for serum titrations is almost identical with that described by Salk.

*Nonspecific Serum Neutralization of Haemagglutination of Influenza Virus.*

Mr. McCrea has continued work on the inhibitory activity of normal rabbit and ferret sera, and has shown that this activity is apparently due to the normal serum gamma globulin fraction, which is capable of neutralizing certain influenza virus strains in a similar way to the neutralization by specific immune gamma globulins. The nonspecific inhibitory activity in normal rabbit sera is readily destroyed by heating at 62°C. for 15-20 minutes, a procedure which leaves specific antibody neutralization comparatively unchanged. This is a useful method of distinguishing between "normal" and immune neutralization in the one serum. Nonspecific activity of other sera is also relatively heat-labile, with one interesting exception in ferret serum, where the nonspecific inhibition of influenza virus strain BEL D has heat stability similar to that of specific antibody.

*Vaccinia and Ectromelia Haemagglutinins.*

The study of the soluble haemagglutinins of vaccinia and ectromelia has been continued by several members of the Virus Department.

Miss Stone and I have made a closer examination of the types of red cell that are agglutinated. The earlier statement that about 50 per cent. of fowls provide susceptible cells, while the other 50 per cent. provide insusceptible ones, requires modification. If strong virus preparations are used, about half the fowls tested give cells that are agglutinated to maximal titre, another third show a range of diminishing agglutinability, while only about a sixth are completely resistant to agglutination, even with concentrated virus. Embryonic cells and those from chicks hatched less than a fortnight are completely insusceptible and show no power to absorb the haemagglutinin.

Of five pigeons tested, two gave susceptible cells agglutinated to titres somewhat lower than the best fowl cells; three were insusceptible. With both fowl and pigeon cells the activities of vaccinia and ectromelia virus preparations were parallel lipid haemagglutinins, also agglutinated the same cells as the virus haemagglutinins, and were completely without action on the cells adult or embryonic, that were not agglutinated by vaccinia.

The only susceptible mammalian cells were those of the mouse. Ectromelia preparations agglutinated these to high titre, the agglutination being inhibited only by specific antiserum. Vaccinia preparations were either inactive, or gave weak agglutination that was inhibited by dilute normal serum. Lipid suspensions also agglutinated mouse cells, and had no definite action on any other of the mammalian types tested.

Using high speed centrifugation, it has been possible to sediment the virus particles and leave the haemagglutinin in the supernatant fluid. It was previously shown that suitable red cells will absorb out the haemagglutinin without reducing the virus titre. These experiments, therefore, establish the soluble nature of the agent, and emphasise its distinction from the other virus haemagglutinins of the mumps-influenza group.

All the preliminary work on vaccinia and ectromelia haemagglutinins was done with material from chorioallantoic lesions. Nagler's earlier work had shown that calf lymph, as ordinarily prepared, contains no haemagglutinin, although its virus content was as high or higher than the chorioallantaic material. A study was, therefore, made by Miss Stone to determine the conditions under which haemagglutinin was produced in vaccinia infection of mammalian tissues. Superficial lesions on the rabbit skin were

shown to contain haemagglutinin in the first five days of their development. At three days there was a well marked relative excess of haemagglutinin in the deeper layers of the skin, of virus in the superficial necrotic tissue. By seven days the detectable haemagglutinin had disappeared and small amounts of antihaemagglutinin were demonstrable. Fresh calf lymph also contains an antihaemagglutinin, and the absence of haemagglutinin can be ascribed with reasonable confidence to the precocious development of antibody capable of inactivating the agglutinin.

*Lipid Haemagglutination.*

Last year it was recognised that the specific haemagglutinin of the viruses of vaccinia and ectromelia was a soluble product distinct from the virus particles. It was further observed that alcoholic extracts of many types of dried normal tissue, when dispersed in saline, gave almost the same type of haemagglutination which, like that due to virus preparations, was effective only against cells from fowls, pigeons and mice, and within each species only against cells from certain individual donors. Cells sensitive to one group of agents were always sensitive to the other.

Miss Stone and Mr. Holden have succeeded in showing that all the properties of the haemagglutinin of normal tissue extracts can be reproduced by suitably dispersed mixtures of purified lecithin and cholesterol in saline. Similar results are also obtainable with purified cephalin, provided it is allowed to oxidise before being tested, with sphingomyelin suitably dispersed with cholesterol, and with cardiolipin (Pangborn), particularly when suspended with lecithin. It seems, therefore, that the cells concerned are susceptible to agglutination by a number of phospholipids, provided these are suitably dispersed.

There is no record of any other type of agglutinating agent which is limited in its action to the unusual range of cells susceptible to vaccinia and lipid haemagglutinins. It, therefore, becomes highly probable from this evidence alone that the activity of the vaccinia haemagglutinin is due to a phospholipid component.

What may be a final proof of the correctness of this hypothesis has recently been obtained by Miss Stone. She has found that the haemagglutinin of vaccinia virus is rapidly inactivated by the lecithinase of *Cl. welchii* toxin, Type A. The action takes place only in the presence of Ca and Mg ions, so is certainly due to the lecithinase. A similar enzymic destruction can be produced by cobra venom which contains another type of lecithinase.

It can, therefore, be stated with some certainty that the haemagglutinin of vaccinia and ectromelia viruses is a relatively stable complex of a phospholipid (almost certainly lecithin) with an antigenically specific product of the virus. It is of interest that the Rockefeller Institute workers found that phospholipids could be obtained from the purified elementary bodies of vaccinia virus. It may well be, therefore, that the soluble haemagglutinin is essentially a product of the disintegration of the virus particles.

*Herpes Simplex.*

Dr. Nagler has continued his investigations on a skin test in human beings as evidence of herpes simplex infections. In the course of these studies he observed that after injection of herpes virus into the yolk sac of chick embryos, the virus infected the amniotic cavity, and after some days the amniotic fluid contained a high content of virus. The volume of fluid recovered after such yolk sac inoculation was usually twice as much as was obtained by direct amniotic inoculation. This fluid from embryos infected with herpes contained a much lower content of protein in relation to the amount of virus present than infected chorioallantoic preparations. As might be expected, heated fluid proved to be a more satisfactory reagent for skin tests than the earlier preparations. In particular, it showed practically no nonspecific reactions in nonherpetic individuals. It is also easy to produce, and is probably the most satisfactory reagent for studies of this sort.

*North Queensland Tick Typhus.*

Major Funder has completed his investigations on the laboratory aspects of this recently discovered rickettsial disease. A comprehensive study has been made of the behaviour of the rickettsia in guinea pigs, rats and chick embryos, as well as by Zinsser's tissue culture methods, and throughout the work comparative studies with Australian strains of murine typhus have been carried on.

In the guinea pig the appearances produced by both types of rickettsia are similar—a low grade fever with moderate to severe scrotal reaction. Cross immunity tests have shown a partial active immunity against the heterologous type.

In rats, the tick typhus strains fail to persist beyond the period of acute infection in sharp contrast to the Australian strains of murine typhus which, like strains studied elsewhere, survive almost indefinitely in the brain of an infected rat.

Tick typhus strains grow fairly readily in the yolk sac of the chick embryo, but it was found very difficult to obtain

rickettsias in sufficient concentration for use in serological tests. The murine strains grew much more freely. By a tedious process of concentration by ether treatment and centrifugation, Major Funder succeeded in obtaining sufficient tick typhus rickettsial emulsion to carry out a full set of quantitative complement fixation reactions with the human and animal sera that were available.

The complement reaction was highly specific as between tick typhus and murine typhus infections, and it was possible to show that sera from the patients diagnosed on clinical and epidemiological grounds as suffering from tick typhus all showed specific complement fixation with the corresponding rickettsial antigen. Sera from the few cases of murine typhus which were diagnosed in North Queensland, showed high fixation with murine antigen, none with tick typhus material.

Throughout this investigation we have been in contact with Colonel Plotz's group at the U.S. Army Medical School, Washington, who have also been working on the disease. The results have been mutually confirmatory, and it is hoped to publish the Australian and American papers on the subject together.

*Publications.*

S. G. Anderson and W. M. Ket:

"The Use of Gamma Globulin in the Prophylaxis of Measles." The Medical Journal of Australia (In the press).

W. I. B. Beveridge:

"Action of Antimony and Some Other Bacteriostatic Substances on Donovania Granulomatis Isolated in the Chick Embryo." The Journal of Immunology (In the press).

W. I. B. Beveridge and F. M. Burnet:

"The Cultivation of Viruses and Rickettsiae in the Chick Embryo." Medical Research Council Special Report Series (In the press).

W. I. B. Beveridge, A. D. Campbell and P. E. Lind:

"Pleuropneumonia-like Organisms in Cases of Nongonococcal Urethritis in Man and in Normal Female Genitalia." Medical Journal of Australia, 1946, 1, 179.

W. I. B. Beveridge, P. E. Lind and S. G. Anderson:

"Mumps I. Isolation and Cultivation of the Virus in the Chick Embryo." The Australian Journal of Experimental Biology and Medical Science, 1946, 24, 15.

F. M. Burnet:

"Haemagglutination by Mumps Virus: Relationship to Newcastle Disease and Influenza Virus." Australian Journal of Science, Vol. 8, Nos. 2, 3, p. 81 (1945).
"Antibody Production in the Light of Recent Genetic Theory." The Australian Journal of Science. (In the press).

F. M. Burnet, H. F. Holden and J. D. Stone:

"Action of Iodine Vapour on Influenza Virus in Droplet Suspension." The Australian Journal of Science, 1945, 7, 125.

F. M. Burnet and J. D. Stone:

"The Haemagglutinins of Vaccinia and Ectromelia Viruses." The Australian Journal of Experimental Biology and Medical Science, 1946, 24, 1.

F. M. Burnet, J. D. Stone and S. G. Anderson:

"An Influenza B Epidemic (Victoria, October, 1945)." The Lancet (In the press).

F. M. Burnet, J. D. Stone and M. Kerr:

"Serological Response to Influenza B Infection in Human Beings: Differentiation of Specific and Nonspecific Type Reactions." The Australian Journal of Experimental Biology and Medical Science (In the press).

J. F. Funder and A. V. Jackson:

"North Queensland Tick Typhus." The Medical Journal of Australia (In the press).

F. P. O. Nagler:

"A Herpes Skin Test Reagent from Amniotic Fluid." The Australian Journal of Experimental Biology and Medical Science (In the press).

J. D. Stone:

"Lipid Haemagglutinins." The Australian Journal of Experimental Biology and Medical Science (In the press).

J. D. Stone and F. M. Burnet:

"The Action of Halogens on Influenza Virus, with Special Reference to the Action of Iodine Vapour on Virus Mists." The Australian Journal of Experimental Biology and Medical Science, 1945, 23, 205.

"The Production of Vaccinia Haemagglutinin in Rabbit Skin." The Australian Journal of Experimental Biology and Medical Science, 1946, 24, 9.

## BIOCHEMICAL DEPARTMENT.

*Cruoralbin and Cruoratin.*

During the period under review work was continued on the derivative blood pigment, cruoralbin. Its prosthetic group cruoratin was separated from the protein and the spectra of some derivatives investigated.

A series of studies was begun on the chromatographic separation of cruoratin from haematin. A large number of white insoluble inorganic salts was used, e.g., oxalates, carbonates, phosphates and sulphates, as well as other adsorbents. The most useful one was found to be barium oxalate, prepared from very dilute solutions of barium chloride and sodium oxalate. Numerous attempts were made to convert cruoratin into bile pigments, but without any success. Determinations of the iron/nitrogen ratio of specimens of cruoratin threw no light on its constitution.

The prosthetic group of "pseudo-haemoglobin" (another "green haemoglobin" related to cruoralbin) was detached from the protein, and proved to be similar to cruoratin. The absorption bands of its derivatives were, however, at a longer wavelength than those of corresponding derivatives of cruoratin. Its solutions were contaminated with small amounts of bili-violins. It has not been found possible to convert the pseudo-haematin into bile-pigments.

Recently, Mr. Holden was able to resume some experiments on the denaturation of the blood-pigment, which had been deferred owing to the war. One interesting feature is the effect of the nature of the derivative of the blood-pigment on the course of the denaturation.

*Photo-electric Colorimetry.*

In view of the increasing use abroad of photo-electric colorimeters in clinical analysis, Mr. Holden decided to construct one in order to gain some personal experience. The one made is similar to the Evelyn, but has a few changes designed to improve its performance. Instead of variable resistances in the lamp circuit the intensity of illumination is controlled by movement of the lamp. To guard the photo-cell and meter from damage through excessive illumination, the colour filters are mounted on a rotatable disc which cannot be removed. The instrument was subjected to a series of consistency tests and found to be satisfactory. Ex-

perience of the instrument over several months has led to these conclusions:

1. Photo-electric colorimetry is not necessarily more accurate than visual colorimetry performed with comparable skill.

2. Photo-electric colorimeters need care in use and maintenance if their accuracy is to be retained.

3. They can give an exaggerated impression of accuracy if used by persons untrained in the principles of their action.

4. Though a photo-electric colorimeter relieves an analyst of the strain of matching tints, the precise handling of such an instrument imposes a mental strain that renders it undesirable to perform more than ten determinations without a short interval of change of occupation.

5. Commercial instruments, especially those made for operation from the mains, should be subjected to examination by an expert before acceptance.

A paper covering this work has been accepted for publication by the Australian Journal of Instrument Technology.

*Preparation of Lipids.*

In association with the work on haemagglutination by lipids carried out in the Virus Department by Miss Stone, Mr. Holden and his assistant carried out an extensive series of preparations providing purified specimens of cephalin, lecithin and sphingomyelin, as well as many fractions of crude tissue extracts.

H. F. Holden:

"Animal Pigments." Annual Review of Physiology, 1945, 14, 601.

"On Cruoralbin and Its Prosthetic Group." The Australian Journal of Experimental Biology and Medical Science, 1945, 23, 255.

"On Some Properties of Cruoratin and Its Relationship to Pseudo-haematin." The Australian Journal of Experimental Biology and Medical Science (In the press).

"A Photo-electric Colorimeter." The Australian Journal of Instrument Technology. (In the press).

*Fermentation of Sugars by Yeasts.*

During part of the year Dr. Gottschalk investigated the mechanism of "selective fermentation" by Sauternes yeast. It is well known that brewer's and baker's yeasts ferment preferentially glucose from invert sugar, whereas Sauternes yeast selects fructose. The first type of selective fermentation is well under-

stood on account of the previous findings that only that fraction (22 p.c. at 25°C.) of d-fructose in solution, which exists in the furanose form, reacts with hexokinase, and that this form has twice the affinity of d-glucose for the enzyme. Accordingly, if glucose and fructofuranose compete for hexokinase, as they do in the fermentation of invert sugar, d-glucose seizes more enzyme molecules, since its higher concentration outweighs the greater affinity of fructofuranose for the common enzyme. With regard to Sauternes yeast it was shown that its capacity to select for fermentation fructose from invert sugar is bound to the intact cell. When invert sugar is fermented by dried preparations of Sauternes yeast, and the fermentation interrupted after the disappearance of two-thirds of the sugar, d-fructose predominates invariably in the residual mixture of glucose and fructose, just as it does with living and dried brewer's yeast. From this it is concluded that the membrane of the intact Sauternes yeast cell is more permeable for fructose than for glucose, and that the selective fermentation of fructose by the living cell results from the preferential permeability of its membrane for fructose, and from the higher affinity of this sugar for hexokinase.

Summarising the above and previous observations, it would appear that in the fermentation of invert sugar by dried preparations of yeasts (baker's, brewer's, Champagne and Sauternes) the relative rates at which d-glucose and d-fructose disappear from the suspension are determined in each case only by the relative concentrations of glucose and of fructofuranose in the suspension and by their relative affinities for hexokinase. Using intact yeast cells the ratio of the relative rates at which the two fermentable sugars pass through the cell wall is an additional factor controlling the relative rates of glucose and of fructose consumption.

Applying the technique of fermentation at low temperature, under which condition the rate of mutarotation is greatly reduced, to mannose it was found that the beta-pyranose modification of the hexose is unfermentable by baker's yeast. This observation, taken in conjunction with the non-fermentability of beta-fructopyranose, previously described from this Institute, indicates that mutarotation is an essential factor in the biological utilization of some naturally occurring carbohydrates.

As in the past five years Dr. Gottschalk was appointed to the part-time staff of the Chemical Department, Melbourne Technical College.

A. Gottschalk:

"Yeast Hexokinase and Its Substrates d-fructofuranose and d-glucose." Nature, 1945, 156, 540.

"Analysis of Sucrose Fermentation by Yeast at 0°C., with Some Remarks on 'Selective Fermentation.'" The Australian Journal of Experimental Biology and Medical Science, 1945, 23, 261.

"The Mechanism of Selective Fermentation of d-fructose from Invert Sugar by Sauternes Yeast." The Biochemical Journal (In the press).

## CLINICAL RESEARCH DEPARTMENT.

*Oriental Schistosomiasis in Australian Servicemen.*

In collaboration with R.A.A.F. medical officers, Miss Williams has made an extensive serological investigation of men exposed to infection with *Schistosoma japonicum* in the Philippines. Complement fixation tests were carried out with an antigen prepared from the livers of snails experimentally infected with *S. spindale.* The antigen had been prepared in India nearly twenty years previously, but had maintained full activity.

The tests showed that of 560 members of the R.A.A.F. who had been exposed to infection, 169 gave a positive complement fixation. Most of these had shown some clinical evidence of infection, and 144 of them were excreting ova in the faeces. Of the 391 giving a negative reaction, only five showed ova in the faeces on repeated examination.

The complement fixation test has, therefore, proved itself of great diagnostic value in this condition, being significantly more sensitive than the other available laboratory procedure, the demonstration of ova in the faeces. Evidence was also obtained that a strongly positive complement fixation reaction (fixation of more than 7 M.H.Ds. of complement) six months after treatment, indicates the persistence of living schistosomes in the body.

*Complement Fixation in Malaria.*

In connection with the investigations carried out at the Army Malarial Research Centre at Cairns, Miss Williams has, during the past two years, applied complement fixation tests to sera from experimental malarial infections. The antigens used were obtained from U.S.A., where they had been prepared from *Pl. gallinaceum* and *Pl. knowlesi* infections. The latter antigen was found to be much more satisfactory for both *Pl. vivax* and *Pl. falciparum* infections in the Cairns cases. Analysis of the results obtained indicates that the complement fixation test has no useful part to play in the laboratory diagnosis of malaria. Positive findings were irregular, and in general were only obtained some time after parasites had been easily demonstrable in blood smears.

*Staphylococcal Osteomyelitis.*

Dr. Nagler has concluded his work on the treatment of experimental osteomyelitis in rabbits with penicillin. By the use of Scherman's method it was found that a condition closely resembling osteomyelitis in man could be produced with regularity. Massive necrotic and hyperplastic lesions were produced in untreated animals.

In the therapeutic experiments, penicillin was given intramuscularly in the form of a suspension in peanut oil and beeswax. If treatment was begun 48 hours after the infection a markedly beneficial effect was evident in most cases, but in a few instances the disease continued to extend. Deaths were almost completely prevented. If the treatment was delayed for six days no significant effect on the bony changes was produced. It seemed probable that for adequate results surgical interference would be necessary in addition to penicillin therapy unless the latter were commenced extremely early. Unfortunately, these investigations could not be carried on further.

*Tests for the Services.*

Blood grouping of Service personnel, which has been carried out at the Institute over the war years, has now been taken over by the Red Cross Blood Transfusion Committee. The change took effect on 1st March, and up to that date 3264 blood groupings had been done by the Institute in the current year.

Miss Williams has continued to carry out complement fixation tests asked for by the Services. Malaria and schistosomiasis tests are mentioned elsewhere. Of the others, 1044 Wassermann reactions, 76 gonococcal, and 310 hydatid complement fixation tests were done during the war. A little over half these tests were for the Services.

*Blood Group Investigations.*

In collaboration with the staff of the Queen Victoria Hospital, Dr. Jakobowicz has studied the various problems that arise from the fact that mother and child may be of different blood group or of Rh type. The relevant data from about 850 deliveries have been analysed, and the results may be summarised as follows:

The already well recognised importance of the Rh factor in the production of haemolytic disease of the new-born is apparent. There is no correlation between the anti-Rh titre of the maternal serum and the severity of haemolytic disease in the child. It appears that A B O incompatibility may be the cause of

haemolytic disease, especially the milder manifestations such as late anaemia. The incidence of icterus gravis is higher than in most previously reported series. The iso-agglutinin titre in the maternal serum, early in pregnancy, is not significantly higher in those cases in which there is a possibility of immunization of the foetus, than it is in those in which mother and child belong to compatible blood groups. At, or more usually shortly after delivery, the maternal iso-agglutinins corresponding to the infant's A or B factor may show a marked rise. It appears to be associated in most cases with the secretor state in the infant, but in some Group O mothers, a nonspecific rise to a much lower degree has been noted in the antibody not corresponding to the infant's antigen.

The effect of cerebrospinal fluid on the interaction between Rh agglutinins and agglutinogens was investigated.

Five human anti-Rh sera containing anti-$Rh_0$ agglutinins were either completely or almost completely prevented from agglutinating Group O $Rh_1$ cells when C.S.F. was used as diluent for sera and red cell suspensions. The inhibitions occurred irrespective of whether the individuals from whom the C.S.F's were taken were Rh-positive or Rh-negative.

An analysis of the factors concerned in this phenomenon has shown that the effect of the C.S.F. is not on the cells, but on the antibody. Cells that have failed to agglutinate in the presence of C.S.F. agglutinate normally with Rh antibody when resuspended in normal saline and, as is the case with so-called blocking antibodies, the addition of serum or albumin overcomes the inhibitory effect of C.S.F. It has not been possible to identify the agent responsible. Artificially prepared fluids containing the main known chemical components of C.S.F. do not mask Rh agglutination, nor does the chemically similar allantoic fluid of the chick embryo. C.S.F. from one rhesus monkey and one rabbit behaved like human C.S.F. The factor in C.S.F. causing the inhibition of Rh agglutinins is heat stable.

L. M. Bryce and R. Jakobowicz:

"The Effect of Cerebrospinal Fluid on the Interaction Between Rh Agglutinins and Agglutinogens." (In the press.)
"Studies of blood factors (A, B and Rh) in Workers and Newborn Babies." (In the press.)

W. P. H. Dakin, J. Connellan and F. E. Williams:

"Asiatic Schistosomiasis: Report of an Outbreak in the R.A.A.F."

F. P. O. Nagler:

"Penicillin Treatment of Experimental Osteomyelitis in Rabbits." The Australian and New Zealand Journal of Surgery. (In the press.)

R. T. Simmons, R. Jakobowicz and G. A. Kelsall:

"The Rh Factor—a Survey of the Sub-types of White Australians." The Medical Journal of Australia, 1945, 2, 493.

## DEPARTMENT OF EXPERIMENTAL MEDICINE (EPIDEMIOLOGY)

*Experimental Epidemiology of Ectromelia of Mice.*

Dr. Fenner has commenced a study of the natural spread of a virus disease of mice, infectious ectromelia. It was recently discovered, as recorded in the last Annual Report, that this disease is very closely related to vaccinia and smallpox, and that haemagglutination techniques can be conveniently applied to its study. Despite the fact that Topley and Greenwood carried out some classic studies on this disease, it was felt that the new techniques now available justified reopening the topic. The nature of the disease which should rightly be called mouse-pox rather than ectromelia, is such that information drawn from such a study might well bear directly on the human problems of smallpox and vaccination.

Preliminary work has been concerned mainly with determining the route of excretion, and the portal of entry of the virus in natural cage infections. Mice can be infected by feeding, but the dose required is about a million times greater than that required to infect by intranasal instillation. Preliminary investigations into the way in which virus reaches the environment suggest that the discharge from skin lesions and the urine during the acute and early convalescent stage of the illness contain active virus. Nasal washings have been positive on only one occasion and virus has not been recovered from the faeces.

Studies on the immunization of mice against ectromelia with vaccinia virus have been continued. The intravenous inoculation of living vaccinia virus gives the best results, and the dose of vaccinia required to protect the majority of inoculated mice against the subsequent inoculation of a concentrated suspension of ectromelia virus is about 800 infective particles.

The haemagglutination-inhibition reaction has been used to follow the development of antibody in infected mice. It has proved to be quite specific and nonspecific inhibitory substances have not been found in normal mouse sera. A technique using

small volumes of reagents has been developed which allows serial determinations of antibody level to be made, the blood being collected from a tail vein into a capillary tube.

A particularly interesting feature of ectromelia infections in mice is the wide variety of clinical syndromes which may follow infection by a standardised dose of virus. The age of the animal appears to play an important part in determining the outcome. Intradermal inoculation of a concentrated virus suspension in the tail of adult mice generally causes a local lesion to develop, the animal remaining healthy. In suckling and immature mice no local lesion develops, and most animals die of acute ectromelia. The reason for this variation in response to infection with varying age is being sought.

*Statistical Work.*

During the year Miss McArthur has carried out the statistical work in connection with a number of investigations by other members of the Institute staff and by workers in the University.

The subjects covered included physiological work on thyrotrophic hormone and on the toxicity of arsphenamine, haematological studies, and a variety of immunological and epidemiological matters derived from the current work of the Institute.

During the summer months an investigation was undertaken by two members of the Dental School staff, Drs. Radden and Sandy, on the possible relation between the presence of exposed nerve endings in carious teeth and the entry of poliomyelitis virus into the central nervous system. This work involved an extensive study of the teeth in poliomyelitis patients in Victoria and Queensland, plus a large control population of normal children. This investigation was made as a joint activity of the Dental School and the Department of Experimental Medicine, and Miss McArthur is at present engaged on the analysis of the results. As far as a preliminary check can indicate, the results do not confirm the American contention that the teeth represent an important portal of entry of the virus.

## LIBRARY.

Our thanks for the gifts of journals and books are due to the following:—Boston Medical School; B.M.A. (New Zealand Branch); The Commonwealth Department of Health; The Council of Scientific and Industrial Research; Miss Danks; Mr. Dobell, F.R.S.; Imperial Chemical Industries, London; Dr. C. H. Kellaway, F.R.S.; The London Hospital; The Medical Research Council; The Middlesex Hospital Medical School; New York Academy of Medicine; New York State Department of Health, Division of

Laboratories and Research; Department of Pathology, University of Oxford; Rockefeller Institute, New York; The South African Institute for Medical Research; U.S. Public Health Service; The University of Harvard, Department of Tropical Medicine; University of Leeds; the University of Pennsylvania, Department of Pathology, and University of Texas School of Medicine.

F. M. BURNET, Director.

**Donations and Subscriptions for the Year Ended 30th June, 1946.**

| | | | |
|---|---|---|---|
| The Truby and Florence Williams Charitable Trust, per Trustees Executors and Agency Co. Ltd. | £1,000 | 0 | 0 |
| The Felton Bequest | 270 | 0 | 0 |
| The Alfred Edments Trust | 100 | 0 | 0 |
| Trustees of the Mackie Bequest | 140 | 0 | 0 |
| Australian Oxygen and Industrial Gases Pty. Ltd. | 25 | 0 | 0 |
| Estate of the late E. M. Carty | 18 | 16 | 7 |
| National Bank of Australasia Ltd., per Lord Mayor's Fund | 10 | 10 | 0 |
| Colonial Sugar Refining Co. Ltd. | 10 | 10 | 0 |
| Mr. and Mrs. H. D. Giddy | 5 | 5 | 0 |
| Miss A. Danks | 4 | 10 | 0 |
| Christy's Products (Aust.) Pty. Ltd. | 2 | 2 | 0 |
| | £1,586 | 13 | 7 |

## DORA LUSH MEMORIAL FUND

| | | | | | | |
|---|---|---|---|---|---|---|
| Funds on hand at 30th June, 1945 | | | | £659 | 7 | 11 |
| Received during year ended 30th June, 1946— | | | | | | |
| Z. Glanville | £10 | 10 | 0 | | | |
| Old Fintonians' Association | 12 | 0 | 0 | | | |
| | | | | 22 | 10 | 0 |
| | | | | £681 | 17 | 11 |

## LIABILITIES

| | | | |
|---|---|---|---|
| General Funds of the Institute at 30/6/1945 .. | £35,290 15 2 | | |
| Less Excess of Expenditure for Year 1945-46.. | 2,997 11 3 | | |
| | | £32,293 | 3 11 |
| Dora Lush Memorial Fund .. .. .. .. | | 681 | 17 11 |
| E. M. Carty Fund as at 30th June, 1945 . | | 4,797 | 3 0 |
| G. C. M. Mathison Fund as at 30th June 1945 .. .. .. .. .. .. .. .. | | 6,500 | 0 0 |
| National Health and Medical Research Council— | | | |
| General and Clinical Research Funds at 30th June, 1945 .. .. .. | 215 15 3 | | |
| Grants for Year .. .. .. | 2,520 18 0 | | |
| | £2,736 13 3 | | |
| Less Expenditure on Materials and Salaries .. .. | 1,981 2 4 | | |
| | | 755 | 10 11 |

NOTE.—No account is taken in the above Statement for the value of apparatus, fittings or equipment of the Institute.

£45,027 15 9

## AUDITOR'S CERTIFICATE.

I beg to report that I have audited the Accounts of the Walter and Eliza Hall Institute of Research in Pathology and Medicine for the year ended 30th June, 1946. I have obtained all the information and explanations required in the course of audit, and I am of opinion that the annexed Balance Sheet and Statement of Receipts and Expenditure are drawn up so as to exhibit a true and correct view of the Institute's affairs, according to the best of my information and the explanations given to me, and as shown by the books of the Institute. (Signed) W. M. JARVIE, F.C.A. (Aust.),
Auditor.

ASSETS

| | | | | | | |
|---|---|---|---|---|---|---|
| Investments on General Account— | | | | | | |
| Australian Consolidated Inscribed Stock | £13,580 | 0 | 0 | | | |
| Melbourne & Metropolitan Board of Works Inscribed Stock, Face Value, £5,800; Cost | 5,743 | 8 | 6 | | | |
| City of Melbourne Inscribed Stock, Face Value, £3,800; Cost | 3,800 | 0 | 0 | | | |
| Melbourne Harbour Trust Inscribed Stock, Face Value, £500; Cost | 500 | 0 | 0 | | | |
| State Savings Bank Credit Foncier Debenture Stock, Face Value, £1,500; Cost | 1,496 | 5 | 0 | | | |
| | | | | 25,119 | 13 | 6 |
| Cash Accounts— | | | | | | |
| Bank of New South Wales, Melbourne | 6,553 | 14 | 8 | | | |
| Funds in London held by Dr. F. M. Burnet | 1,882 | 10 | 0 | | | |
| Agent-General, London | 24 | 14 | 7 | | | |
| English, Scottish and Australian Bank, North Melbourne Trust Account | 100 | 0 | 0 | | | |
| Petty Cash Account | 50 | 0 | 0 | | | |
| | | | | 8,610 | 19 | 3 |
| E. M. Carty Fund Investments— | | | | | | |
| Australian Consolidated Inscribed Stock, Face Value, £4,490; Cost, | 4,490 | 0 | 0 | | | |
| Melbourne and Metropolitan Board of Works, Inscribed Stock, Face Value, £250; Cost | 250 | 0 | 0 | | | |
| Fixed Depost, Bank of New South Wales | 54 | 15 | 0 | | | |
| Bank of New South Wales, Current Account | 2 | 8 | 0 | | | |
| | | | | 4,797 | 3 | 0 |
| G. C. M. Mathison Trust Fund Investments— | | | | | | |
| Australian Consolidated Inscribed Stock, Face Value £6,500; Cost | | | | 6,500 | 0 | 0 |
| | | | | £45,027 | 15 | 9 |

| | | | | |
|---|---|---|---|---|
| To Balance to Credit of Current Account—Bank of New South Wales, 1st July, 1945 . . . . . | | | £5,860 | 1 6 |
| „ Grants— | | | | |
| The Walter and Eliza Hall Trustees . . . | £3,200 0 0 | | | |
| The University of Melbourne . . . . | 750 0 0 | | | |
| „ The National Health & Medical Research Council | | | | |
| Expended on General and Clinical Research . . . . . | 1,981 2 4 | | | |
| Expended on Virus Research . . . . . | 4,525 0 0 | | | |
| Refund of Pay Roll Tax . . . . . | 101 4 10 | | | |
| | | £10,557 7 2 | | |
| „ Donations and Subscriptions as per attached statement . . . . . | | 1,586 13 7 | | |
| „ Fees Received for Services . . . . . | 1,582 19 5 | | | |
| „ Refund from Commonwealth Serum Laboratories for Influenza Vaccine Production Work | 1,716 19 3 | | | |
| | | 3,299 18 8 | | |
| „ Income from Investments as listed . | | 1,023 15 5 | | |
| „ Proceeds from Sale of Publications | | 99 8 0 | | |
| „ Surplus on Sale of £5000 Aust. Consol. Insc. Stock . . . . . | | 21 17 6 | | |
| Income for Year Ended 30th June, 1946 . . . . . | | | 16,589 | 0 4 |
| „ Donations to Dora Lush Memorial Fund . . . . . | | | 22 | 10 0 |
| „ Bank of New South Wales Fixed Deposit Repaid . . | | | 63 | 16 9 |
| „ Sale of Aust. Consol. Insc. Stock . . . . . | | | 5,000 | 0 0 |
| „ National Health and Medical Research Council— | | | | |
| Balance of Grant for General and Clinical Research as yet unexpended . . . . . | | | 539 | 15 8 |
| Note: Total grant received for the year was £2,520 18 0 | | | | |
| | | | £28,075 | 4 3 |

| | | | |
|---|---|---|---|
| By National Health and Medical Research Council Grants— | | | |
| General and Clinical Research— | | | |
| Salaries and Materials . . . . . 1,981 2 4 | | | |
| Virus Research— | | | |
| Salaries and Materials . . . . . 4,674 13 6 | | | |
| | £6,655 15 10 | | |
| „ G. C. M. Mathison Fellowship— | | | |
| Salary Paid . . . . . £575 0 0 | | | |
| Income of Trust Fund 211 5 0 | | | |
| | £363 15 0 | | |
| „ E. M. Carty Fellowship— | | | |
| Salary Paid . . . . . 500 0 0 | | | |
| Income of Trust Fund 137 16 10 | | | |
| | 362 3 2 | | |
| | | £7,381 | 14 0 |
| „ Salaries and Wages— | | | |
| Professional . . . . . | 4,326 16 9 | | |
| Secretarial . . . . . | 580 15 0 | | |
| Assistants . . . . . | 2,244 7 8 | | |
| | | 7,151 | 19 5 |
| (Total Salaries and Wages paid by the Institute for year ended 30/6/46, £13,548/3/9.) | | | |
| „ Materials . . . . . | | 1,132 | 19 7 |
| „ Apparatus . . . . . | | 426 | 16 11 |
| „ Printing and Stationery . . . . . | | 87 | 15 8 |
| „ Postage, Telephone, Cables, Etc. . . . . | | 129 | 5 5 |
| „ General Expenses . . . . . | | 653 | 10 4 |
| „ General Maintenance . . . . . | | 1,694 | 15 11 |
| „ Library Purchases . . . . . | | 176 | 1 1 |
| „ Pay Roll Tax . . . . . | | 312 | 13 1 |
| „ Fittings and Equipment . . . . . | | 439 | 0 2 |
| Total Expenditure for year ended 30/6/46 . . . . | | 19,586 | 11 7 |
| „ Funds in London to Cover Travelling Expenses and Projected Equipment Purchases by Dr. F. M. Burnet . . . . . | | 1,882 | 10 0 |
| „ Petty Cash Account, E.S. & A. Bank . . . . | | 50 | 0 0 |
| „ Balance to Credit of Current Account, Bank of New South Wales . . . . . | | 6,556 | 2 8 |
| | | £28,075 | 4 3 |